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Vaccinia Virus-Encoded eIF-2α Homolog Abrogates the Antiviral Effect of Interferon

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One of the well-established antiviral mechanisms induced by interferon (IFN) is the inhibition of initiation of protein synthesis due to the phosphorylation of the small subunit of eukaryotic initiation factor 2α (eIF-2α) by the interferon-induced P1 kinase (*1*, *2*). Vaccinia virus (VV) has been shown to be resistant to IFN (*3*, *4*) and capable of rescuing IFN-sensitive viruses from the effects of IFN by somehow reducing the level of eIF-2α phosphorylation (*5*, *6*). The complete nucleotide sequence of the VV genome (*7*, *8*) has revealed an 88-amino-acid long open reading frame (ORF), designated as K3L, which has 28% identity to eIF-2α over an 87-amino-acid region (Fig. 1). This report presents the generation of a VV mutant, vP872, in which the K3L ORF has been specifically deleted in order to evaluate the relevance of this VV gene to the IFN-resistant phenotype (*3*, *4*). Monitoring both virus-induced protein synthesis and viral yields in mouse L929 (or human MRC-5) cells pretreated with increasing concentrations of mouse α/β (or human lymphoblastoid) IFN suggests a correlation between the presence of the K3L gene and the IFN-resistant phenotype.

The amino acid sequence of the K3L ORF identified in the Copenhagen strain of VV (VC-2) (*7*, *8*) is compared to the amino acid sequence of eIF-2α (*9*) in Fig. 1. The VV K3L ORF has the potential to encode a 10.5-kDa protein, whereas eIF-2α has a calculated molecular mass of 36.1 kDa. Significantly, the highly homologous 87-amino-acid overlap region spans the amino-terminal portion of eIF-2α and includes the serine residue (amino acid 51) known to be phosphorylated by the interferon-induced P1 kinase (*10*).

Generation of the VV deletion mutant, vP872, by *in vivo* recombination (*11*) was accomplished using deletion plasmid pK3Lgpt, wherein the *Escherichia coli* hypoxanthine–guanine phosphoribosyl transferase (*Ecogpt*) gene completely replaces the K3L coding region in the wild-type virus, VC-2. Southern blot analysis of viral DNA derived from the wild-type and K3L deletion mutant vP872 was used to confirm the specific deletion of the K3L gene. DNA from wild-type (Fig. 2; lanes 1, 3, 5, and 7) or deletion mutant vP872 (Fig. 2; lanes 2, 4, 6, and 8) was digested with *Hin*dIII, fractionated on an agarose gel, and transferred to a nylon membrane for analysis by hybridization. The resultant autoradiograms obtained by hybridization with radiolabeled total wild-type DNA (VAC), K3L-specific probe (K3L), *Hin*dIII K-specific probe (*Hin*d K), or *Ecogpt*-specific probe (GPT) are shown in Fig. 2. Hybridization with total radiolabeled wild-type DNA demonstrated that the only *Hin*dIII fragment altered during the deletion of the K3L ORF was the 4.6-kbp *Hin*dIII K fragment (Fig. 2; lanes 1 and 2). The substitution of the K3L ORF with *Ecogpt* using plasmid pK3Lgpt results in the introduction of two additional *Hin*dIII sites flanking the *Ecogpt* expression cassette. Thus *Hin*dIII digestion results in the generation of a 2.8- and a 1.5-kbp *Hin*dIII K subfragment (indicated by asterisks) in the deletion mutant vP872 DNA (Fig. 2; lane 2). These results were confirmed by probing the DNA with purified, radiolabeled *Hin*dIII K fragment (Fig. 2; lanes 5 and 6).

Confirmation of the specific deletion of the K3L ORF by substitution with *Ecogpt* is further provided by hybridization with radiolabeled K3L-specific and *Ecogpt*-specific probes (Fig. 2; lanes 3, 4 and 7, 8, respectively). As shown by the autoradiograms, with wild-type DNA the 4.6-kbp *Hin*dIII K fragment hybridized with the K3L-specific probe (Fig. 2; lane 3), and no hybridization occurred with the *Ecogpt*-specific probe (Fig. 2; lane 7). Conversely, the DNA obtained from the K3L deletion mutant vP872 did not hybridize to the K3L-specific probe (Fig. 2; lane 4), but the *Ecogpt*-specific probe did hybridize to a 1-kbp *Hin*dIII fragment (Fig. 2; lane 8), a result consistent with the substitution of the K3L ORF with a 1-kbp *Ecogpt* expression cassette flanked by *Hin*dIII restriction sites.

To assess the effect of the K3L-specific deletion on protein synthesis in IFN-treated VV-infected cells, wild-type VC-2 or the deletion mutant vP872 were inoculated onto L929 cell monolayers which had been pretreated for 24 hr with increasing concentrations of

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K3L      MLAFCVSLPNAGDVIKGRVYE-KDYALYIYLFDYPHSEA-ILACSVKMHMQRYVEY  54
IF2A     MPGLSCRFYQHKFPEVEDVVMVNVRSIAEMGAYVSLLEYNNIEGMILLSELSRRRIRSIN-  60
                                                  *
K3L      RDKLVGKTVKVKVIRVDYTKGYIDVNYKRMCRHQ                       87
IF2A     KLIRIGRNECVVVIRVDKEKGYIDLSKRRVSPEEAIKCEDKFTKSKTVYSILRHVAEVLE  120
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FIG. 1. Alignment of the entire amino acid sequence of the K3L ORF (88 amino acids) from the Copenhagen strain of vaccinia virus (VC-2) (7 with the first 120 amino acids of eIF-2α (9). This alignment has been optimized by gap insertions. The asterisk at amino acid 51 of the eIF- sequence denotes the serine residue which is phosphorylated by the interferon-induced P1 kinase (10). Amino acid homology was obtain using the FASTP (27) program of PCGENE against the Swisprot database release 11.0 (IntelliGenetics, Inc., Mountain View, CA).

mouse α/β IFN. At 7 hr postinfection, cells were pulsed for 1 hr with [^{35}S]methionine and then harvested. Aliquots containing equal quantities of total protein from each sample were fractionated by SDS–PAGE, and radiolabeled proteins were visualized by fluorography as shown in Fig. 3. Uninfected cell controls showed no effect of IFN on host cellular protein synthesis even at IFN concentrations of 1000 IRU/ml (Fig. 3; lanes A–C). Viral-induced protein synthesis in wild-type VC-2-infected cells was largely resistant to interferon, although a slight diminution was noted at IFN concentrations of 500 IRU/ml or greater (Fig. 3; lanes D–H). These results are consistent with previously described results for wild-type VV-infected cells treated with IFN (3, 4). In marked contrast, the specific deletion of the K3L ORF greatly enhanced the sensitivity of viral-induced protein synthesis to IFN pretreatment (Fig. 3; lanes I–M). IFN concentrations as low as 10 IRU/ml significantly reduced the level of virus-induced protein synthesis in vP872-infected cells (Fig. 3; lane J). Viral-induced protein synthesis in IFN-treated vP872-infected L929 cells was almost completely inhibited at IFN concentrations of 100 IRU/ml and higher (Fig. 3; lanes K–M). Sensitivity of viral protein synthesis to IFN observed in vP872-infected cells is not due to the expression of the *Ecogpt* gene, since a VV recombinant with an intact K3L ORF containing the identical *Ecogpt* expression cassette as vP872 displayed an IFN-resistant phenotype similar to wild-type VV (data not shown).

Consistent with the reduction in protein synthesis, viral replication was also found to be highly sensitive to the presence of IFN with the K3L deletion mutant vP872 (Fig. 4). Replication was reduced by almost 100-fold at lower IFN concentrations (10 and 100 IRU/ml) and fell below residual input virus levels at higher IFN concentrations (500 and 1000 IRU/ml). In contrast, the replication of wild-type vaccinia virus was reduced by less than a log even at the highest concentration of IFN tested (1000 IRU/ml). In the absence of IFN both wild-type and deletion mutant virus gave similar yields (Fig. 4), consistent with the K3L gene being nonessential for viral replication in tissue culture (12).

These results strongly suggest that the VV K3L ge is involved in the IFN-resistant phenotype describ previously for VV (3, 4) and provides yet anoth glimpse into vaccinia/host cell interactions. Previous three additional VV-encoded gene products were d fined which may also enhance the pathogenicity of t virus (13). The VGF (14), SERPINS (15), and the co plement-binding proteins (16), in addition to the K3 encoded function, all potentially influence the replic tion of VV *in vivo* and the control of the virus by specif and nonspecific immune effector functions.

The mechanism by which the K3L gene produ confers IFN-resistance remains to be detailed. It m have similarity to previously reported mechanism which demonstrated that (1) an exogenous source eIF-2 could rescue protein synthesis in VSV-infecte L929 cell lysates (17) or (2) an exogenous source eIF-2α was able to overcome the inhibitory effects eIF-2α phosphorylation and enable the replication of mutant form of adenovirus type 5, which fails to e press virus-associated RNA (18, 19). Of significanc the plasmid-expressed exogenous source of eIF-2 contained an amino acid substitution of a serine to a alanine at position 51, thus preventing the phosphor lation at this position, an event highly correlated wit translational repression (18). The VV K3L ORF does n contain a serine residue at the equivalent position. In terestingly, the activity of certain cellular protein k nases is inhibited by pseudosubstrates (either withi the regulatory subunit of the protein itself or as a syn thetic peptide) which resemble the kinase substrate but lack the phosphorylation site (20, 21, 22). The K3L specified gene product may therefore impart IFN resis tance by binding competitively to the P1 kinase t block cellular eIF-2α phosphorylation. It is of interes that transcription of the K3L region occurs at earl times postinfection (23), especially in light of previous findings which demonstrated that the VV-mediated res cue of VSV from the antiviral effects of IFN require early VV RNA synthesis (24).

The data presented here show that the VV K3L gen plays an integral role in the resistance to interferon b the Copenhagen strain of VV. The WR strain of VV alsc

FIG. 2. Southern blot analysis of *Hin*dIII-digested DNA from deletion mutant vP872 (lanes 2, 4, 6, and 8) and from wild-type virus VC-2 (lanes 1, 3, 5, and 7). Lanes 1 and 2 were hybridized with total VC-2 DNA (VAC). Lanes 3 and 4 were probed with a PCR-derived fragment exclusively containing the K3L ORF (K3L). Lanes 5 and 6 were hybridized with isolated *Hin*dIII K fragment (*Hin*d K) and lanes 7 and 8 were probed with a PCR-derived fragment containing the *Ecogpt* gene (GPT). The arrowhead indicates the *Hin*dIII K fragment of the wild-type VV and asterisks indicate the two *Hin*dIII fragments generated when the *Ecogpt* insert is liberated from the K3L deletion mutant vP872. Size markers in kilobasepairs are provided in the left-hand margin of the figure. Deletion mutant vP872 was engineered in the following manner. Both the upstream (5') and the downstream (3') sequences relative to the K3L ORF were derived by PCR. The resultant fragments were digested with the appropriate restriction enzymes and ligated together into pBS-SK+ (Stratagene, La Jolla, CA) vector. The resultant plasmid was designated pK3LA. A 1-kb *Hin*dIII fragment containing the *E. coli gpt* (*Ecogpt*) gene (ATCC No. 37145) juxtaposed 3' to a 300-bp fragment derived from the promoter region of the VC-2 hemorrhagic gene (*7, 8, 26*) was inserted into the unique *Hin*dIII site of pK3LA. The resultant plasmid, designated pK3Lgpt, was used in standard *in vivo* recombination experiments (*11*) with wild-type VC-2 as the rescue virus. Potential K3L-minus mutants containing the *Ecogpt* gene under the control of the hemorrhagic promoter were selected by plating in the presence of selective medium containing mycophenolic acid (*29, 30*). Plaque-purified populations were confirmed for the loss of the K3L ORF by their inability to hybridize to a K3L-specific probe. Viral DNA was extracted from purified virions as described previously (*31*), digested with *Hin*dIII, and fractionated on a 0.8% agarose gel. The gel was treated for transfer of DNA as described previously (*32*). The DNA was transferred to Hybond-N (Amersham Corp., Arlington Heights, IL) and immobilized by uv-irradiation according to the manufacturer's specifications. Prehybridization, hybridization, and visualization were performed as previously described (*11*).

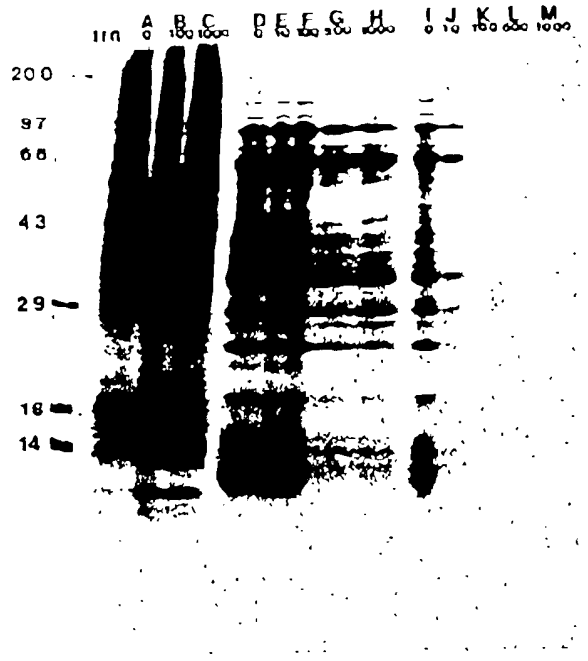

FIG. 3. Effect of IFN on viral-induced protein synthesis in wild-type VV and deletion mutant vP872-infected L929 cells. L929 (ATCC No. CCL1) cell monolayers were pretreated for 24 hr with 0, 10, 100, 500, or 1000 IRU/ml of mouse α/β IFN (Lee BioMolecular Research Laboratories, Inc., San Diego, CA). Cell monolayers were mock-infected (lanes A–C), infected with wild-type virus VC-2 (lanes D–H), or deletion mutant vP872 (lanes I–M) at an m.o.i. of 100. ^{14}C-radiolabeled protein size standards (Bethesda Research Laboratories, Gaithersburg, MD) are indicated on the left. Cell lysates were prepared as follows. After a 1-hr adsorption period, the inoculum was removed and the monolayers were washed. Two milliliters of methionine-free modified Eagle's medium (ICN Flow, Costa Mesa, CA) containing 2% dialyzed FBS was applied to the monolayers. At 7 hr postinfection, medium was aspirated and 2 ml of the same medium supplemented with 25 μCi/ml [^{35}S]methionine (E. I. DuPont de Nemours & Co. Inc., Boston, MA) was applied to the monolayers. At 8 hr postinfection, the medium was aspirated from the monolayers and washed with PBS. Lysates were prepared by three cycles of freeze-thawing followed by clarification of the lysate. Total protein concentrations of the lysates were determined using the Bio-Rad Protein Assay kit (Bio-Rad Laboratories, Richmond, CA), which is based on the Bradford method (*33*). Equivalent quantities of total protein from each sample were fractionated by SDS–PAGE. The gel was fixed, washed, and treated with 1 *M* sodium salicylate. The gel was then dried and exposed to Kodak XAR-2 film for visualization.

has a K3L gene (*25*) which shares homology with eIF-2α and differs from its Copenhagen homolog by three base changes, two of which are conservative at the amino acid level. Preliminary results have shown that disruption of K3L gene expression in the WR strain also results in increased sensitivity to interferon (unpublished observations). Further, both vaccinia strains, Copenhagen and WR, are resistant to human lymphoblastoid IFN on human cells. K3L deletion mutants

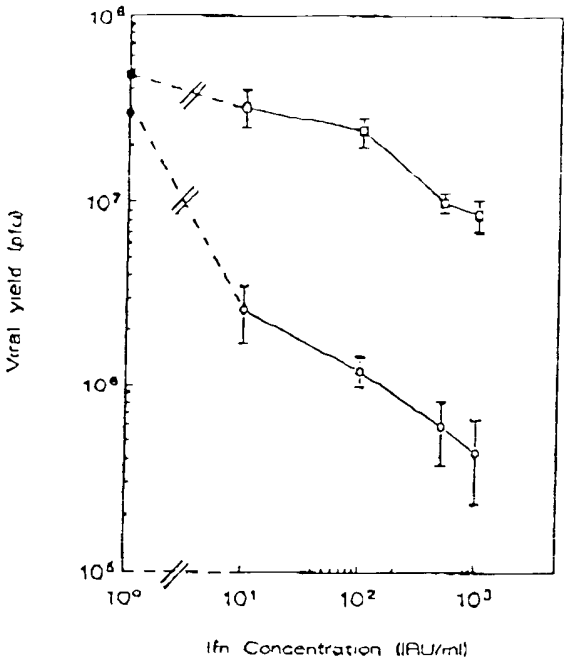

FIG. 4. Effect of IFN treatment on viral replication of wild-type and vP872-infected L929 cells. Each point represents the average of six plates from a representative experiment using (□) wild-type virus VC-2 or (○) deletion mutant vP872. Viral yields in the absence of interferon are indicated by solid markers on the ordinate. A dashed line with hatchmarks is drawn from these points to the corresponding graph for continuity. Samples were treated identically as those for Fig. 3 except that following the adsorption period, 2 ml complete MEM was added and harvest was at 24 hr p.i. Lysates were prepared as per Fig. 3 without clarification and plated onto monolayers of Vero cells (*11*). Samples were inoculated in duplicate and plated in triplicate. Plates harvested immediately following the adsorption period had an average yield of 3.6×10^6 PFU.

from both strains are rendered sensitive to human lymphoblastoid IFN when tested on human MRC-5 cells (data not presented). Studies are currently underway to further define the role of the K3L ORF in the IFN-resistant phenotype *in vivo*. Additional studies are in progress to further define the molecular mechanisms by which VV evades the antiviral effects of interferon.

These findings may also have practical relevance in the use of VV-based vaccine candidates as immunizing agents (*26*), since a vector sensitive to IFN may provide a means for drug intervention upon the unlikely event of an adverse vaccination reaction.

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CHAPTER 12

Virus–Host-Cell Interactions

David M. Knipe

By definition, viruses are unable to replicate on their own but must enter a host cell and use the host-cell macromolecular machinery and energy supplies to replicate. During their replication within cells, viruses may exploit host-cell molecules and processes at the expense of the host cell. These injurious effects of viral replication in cells are one of the basic causes of viral disease. Therefore, precise knowledge of the mechanisms by which viruses replicate in specific tissues, spread, and cause disease must come, in part, from studies of the intracellular replication of the virus. Over the past 40 years, increasing understanding of the mechanisms of viral replication has emerged from biochemical and cell biological studies of virus replication in cultured cells. Studies of viral pathogenesis have recently expanded to attempt to define the molecular events occurring in different cell types during the series of stages that define viral pathogenesis within a host organism. This chapter will focus on the interactions of viruses with an individual host cell. Chapter 10 discusses the events that lead to (a) spread of a virus from one cell to another within a host organism, (b) induction of disease, and (c) spread within the environment.

Virus infection of a cell can lead to any of several possible outcomes. First, a nonproductive infection can occur, in which viral replication is blocked, and the host cell may or may not survive. Following the

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nonproductive infection, the viral genome may be lost from the cell. Alternatively, the viral genetic information may become integrated as DNA in the cellular genome or may persist as episomal DNA in these surviving cells. If the growth properties of the cell are altered to make it oncogenic, this would constitute an oncogenic transformation event (see Chapter 13). The virus may become dormant with little viral gene expression, and a latent infection results (see Chapter 11). Second, a productive viral infection may result in which the host cell dies and lyses. Third, the cell may survive and continue to produce virus at a low level, resulting in a persistent infection (see Chapter 11). Which of these possible scenarios becomes the outcome of viral infection of a host cell is determined by the nature of the interactions between the virus and the host-cell constitutents. For example, a nonproductive infection may result if a host-cell component necessary for viral replication is not present. One of the main goals of this chapter is to describe the types of interactions between virus-encoded macromolecules and the host cell which may define the ultimate outcome of a virus infection. This chapter will examine (a) the molecular and cell biological events that allow viral replication, (b) the ways in which viruses modify their host cells to promote their own replication, and (c) the kinds of mechanisms that may have evolved in cells to prevent virus infection. The types of experimental approaches utilized to obtain evidence for specific virus–host interactions will also be discussed.

The study of virus–cell interactions really started with the growth of viruses in cultured cells (47). Although infection of host organisms had given some indication of cell death resulting from viral infection, there was little clear evidence of other effects of viruses on the host cell prior to the infection of cultured cells and the identification of cytopathic effect (CPE) of viruses on cells (46). The elucidation of viral replication strategies in the 1950s and 1960s provided the broad outlines of virus replication. More recently, better probes for nucleic acids and proteins have allowed more precise descriptions of the molecular events of viral replication in a host cell. The techniques of molecular genetics and cell biology have also defined the specific host-cell molecules and cellular compartments with which viral-encoded molecules interact. This provides one of the second themes of this chapter. In addition to defining the events of viral replication, molecular and cell biological studies of virus replication have utilized viruses as probes of the eukaryotic host cell. Viruses often poke their way into host-cell metabolism in such a subtle way that understanding viral replication can provide knowledge of critical metabolic events of the host cell. Thus, viruses often mimic their host cell (or have evolved from the host cell; see Chapter 9) in such a way that viruses frequently provide a prototype mechanism for a specific molecular problem. In many cases, the initial evidence for a specific molecular event has come from the study of viruses and their intracellular replication processes.

In addition to classifying virus–host-cell interactions in terms of the final outcome of the infection, virus–cell interactions can also be described in molecular terms with regard to individual replication events. For example, the effects of viruses on the host cell can be mediated by addition or substitution of a virus-specific macromolecule to a cellular complex or structure. Alternatively, the virus may mediate a covalent or noncovalent modification of a host-cell molecule. Virus infection may cause a disassembly or rearrangement of a host-cell complex or structure, or virus infection may lead to the assembly of a new infected cell-specific complex or structure in the infected cell.

CYTOPATHIC EFFECTS OF VIRUS INFECTION

One of the classic ways of detecting virus replication in cells is the observation of changes in cell structure, or CPE, resulting from virus infection. Some of the most common effects of viral infection are morphological changes such as (a) cell rounding and detachment from the substrate (Fig. 1), (b) cell lysis, (c) syncytium formation (Fig. 2), and (d) inclusion body formation (Fig. 3). The occurrence of cell morphological changes resulting from CPE has even led to classification schemes for viruses. Enders (46) proposed classifying viruses into the following groups: (a) those causing cellular degeneration; (b) those causing formation of inclusion bodies and cell degeneration; and (c) those causing formation of multinucleated cells or syncytial masses and degeneration, with or without inclusion bodies. However, as described in Chapter 2, other classification schemes based on virion and genome structure and modes of replication have provided much better ways of classifying viruses.

The CPE of viruses on cells has also been called *cell injury*. These terms tend to emphasize the pathology of the host cell; however, from a virologist's point of view, we will see that many of the host-cell alterations by virus infection can now be explained as changes in the host cell that permit necessary steps in viral replication. Thus, many of the CPEs or cell injuries are secondary effects of the virus doing what it needs to do to replicate and are not simply toxic effects of viral gene products on the host cell. However, there are some viral gene products that cause toxic effects to the host with no known purpose. For example the adenovirus virion protein named *penton protein* causes a rapid CPE on monolayer cells (233). The role of this effect on the host cell is unknown. In contrast, some virus–host-cell interactions cause no apparent injury to the host cell.

A number of recent reviews and monographs have

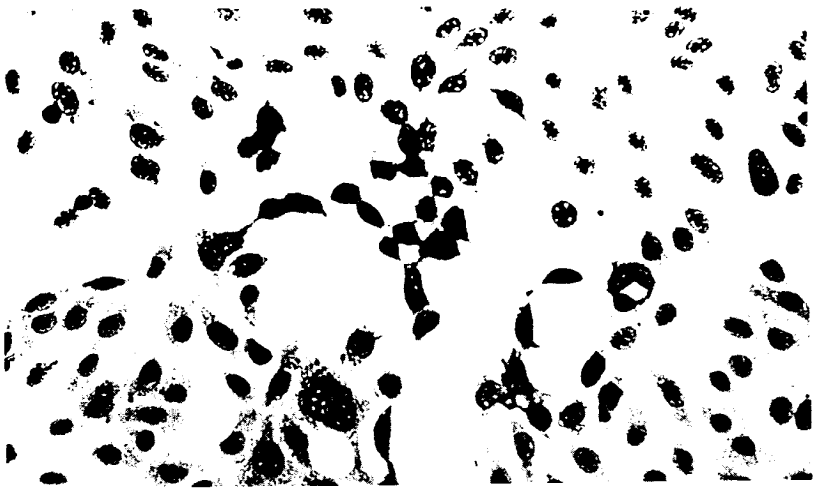

FIG. 1. Cytopathic effect (CPE) due to virus infection. The center portion of the figure shows monkey (Vero) cells rounding up and detaching from the substrate after infection with herpes simplex virus type 1 (HSV-1). A normal monolayer of cells is visible around the focus of CPE. The cells were fixed with methanol and stained with Giemsa stain. (Micrograph courtesy of M. Kosz-Vnenchak.)

examined both the various aspects of cytopathology and the causes of cell death (see, e.g., refs. 56, 110, and 202). The reader is referred to these for detailed discussion and references on these issues. Determining the primary cause of death of a cell resulting from viral infection can be a complex and difficult issue because of the many events occurring within the infected cell. The focus of this chapter will be an examination of the stages of viral replication within the host cell where interactions between viral gene products and the host cell take place.

VIRUS INTERACTIONS WITH CELL UPTAKE MECHANISMS

Viruses must enter the host cell to replicate. Therefore, they must cross the cell plasma membrane to gain access to the cellular synthetic machinery in the nucleus and/or cytoplasm. Virus entry into the host cell has been divided into two events: (i) binding to cell-surface receptors and (ii) penetration of the plasma membrane. These two events will be discussed separately.

Binding to Cell-Surface Receptors

The first event in viral infection of the host cell is binding of the virus to the cell surface. The cell-surface molecule with which the virus first interacts or binds is called the *cell receptor*. It has been difficult to define the physiological receptor for viruses for several reasons: (a) a virus may bind specifically or nonspecifically to a number of surface molecules; (b) a virus is a large ligand that can interact with a large surface area on the cell, thereby giving numerous cellular molecules that may cofractionate with a virus–receptor complex;

FIG. 2. Syncytium formation due to virus infection. The *arrow* indicates a cluster of nuclei within a syncytium formed after infection of monkey cells with a syncytia-forming mutant strain of HSV-1. The cells were fixed with methanol and stained with Giemsa stain. (Micrograph courtesy of M. Kosz-Vnenchak.)

FIG. 3. Inclusion body formation in infected cells. The *arrow* indicates an intranuclear eosinophilic inclusion in a cell infected with HSV-1. The inclusion is surrounded by a clear halo. The cells were stained with hematoxylin and eosin. (Micrograph courtesy of M. Kosz-Vnenchak.)

or (c) a virus may have alternate receptors on individual or different cells. Nevertheless, specific receptors have been defined for several viruses. Several approaches have been used to attempt to identify virus receptors: (a) use of specific chemical compounds to compete for, and block virus binding and infection of cells; (b) use of monoclonal antibodies specific for cell-surface proteins to block virus binding; (c) enzyme treatment of the cell surface to remove receptor activity; (d) purification of virus–receptor complexes; (e) use of anti-idiotypic antibodies to purify receptors; (f) gene transfer of receptor activity to receptor-negative cells, as well as cloning of transferred gene sequences; and (g) correlation of receptor activity with expression of a specific molecule on the cell surface.

Viruses utilize a wide variety of cell-surface molecules as their receptors in that they can use protein molecules (see below), carbohydrates (66), or glycolipids (136) as their cellular receptors. Some receptors are specific molecules such as the CD4 protein molecule, which serves as the receptor for human immunodeficiency virus (HIV) on T lymphocytes (40,102). Other receptor molecules are widely distributed molecular moieties such as sialic acid, which serves as the receptor for influenza (66), or heparan sulfate, which serves as the initial cell receptor for herpes simplex virus (HSV) (243). Because a molecule such as CD4 is restricted to certain types of cells, this receptor activity is restricted to a specific tissue. Other examples of known tissue-specific receptors are (a) the C3d complement receptor on B cells, which serves as the receptor for Epstein–Barr virus (54,55,152), and (b) the acetylcholine receptor, which may serve as a receptor for rabies virus (124). Thus, as their receptors, viruses can utilize cell-surface molecules that normally serve the host cells as receptors for other molecules. In this way, viruses are utilizing the normal host-cell pathways for internalization of molecules or extracellular signals.

Receptors may be species-specific also. For example, the poliovirus receptor is found only on primate cells and not on other mammalian cells (141). The block to poliovirus replication in murine cells is only at the surface because viral RNA introduced into murine cells is infectious (82). Using sensitivity to poliovirus infection as a screen, a human gene encoding a poliovirus receptor was transferred into murine cells (143). By identifying the human DNA sequences that correlated with receptor expression, the poliovirus receptor gene was recently isolated from these cells (144). The predicted amino sequence for the encoded protein indicates that the receptor is an integral membrane protein with characteristics of members of the immunoglobulin superfamily of proteins. Interestingly, the receptor for another picornavirus, human rhinovirus, is intercellular adhesion molecule-1 (ICAM-1) (70,222), which is also a member of the immunoglobulin superfamily of proteins.

Interaction of a virus with a cellular receptor represents the first interaction between virus and host cell. Obviously, if the virus cannot bind to the host cell, infection cannot be initiated, and minimal effects on the cell are likely to result. Even if the virus cannot enter the cell, it is conceivable that virus binding could exert an effect on the host cell. For example, it has been hypothesized that binding of Epstein–Barr virus to the surface of a B lymphocyte can initiate the activation of the B cell (65,88). Thus, the virus particle could act as a ligand to initiate the transfer of a signal to the interior of the host cell.

Entry into the Host Cell

After binding to its surface receptor, a virus must cross the plasma membrane to replicate. Two general pathways have been defined for virus entry, namely, surface fusion and receptor-mediated endocytosis. These will be described separately.

Surface Fusion

Some enveloped viruses, notably the paramyxoviruses and the herpesviruses, are capable of fusion with the cell plasma membrane at the cell surface. Binding of the virus to the cell-surface receptor leads to fusion between the virion lipid envelope and the cell plasma membrane. This releases the viral nucleocapsid into the cytoplasm of the host cell, effecting penetration of the host-cell plasma membrane. Surface fusion of enveloped viruses is promoted by virion surface proteins.

For Sendai virus (for example), the fusion (F) protein is synthesized as a precursor molecule that is unable to mediate fusion. Extracellular proteases cleave the precursor molecule after it is localized to the cell surface or assembled onto the virion surface. This cleavage exposes a hydrophobic amino terminus of the F protein (86), which promotes fusion between the lipid envelope of the virus and cell plasma membrane.

Endocytosis

Some viruses, notably Semliki Forest virus and influenza virus, enter cells by an endocytotic pathway that exploits a normal cellular pathway for uptake of materials bound to cell-surface receptors (212). Following binding of the viruses to the cell surface, a clathrin-coated invagination of the plasma membrane, called a *coated pit*, is formed. A vesicle pinches off with the virion particle inside. Acidification of the interior of the vesicle is then promoted by a proton pump in the membrane. As the pH drops, glycoproteins on the virion surface undergo a conformational change, causing exposure of a hydrophobic portion of a virion surface protein. This hydrophobic region promotes fusion between the virion lipid envelope and the vesicle membrane, releasing the nucleocapsid into the cytoplasm. At this point, the viral genetic material has entered the host cell. For viruses lacking a lipid envelope, this pathway may also be used, but entry cannot be via membrane fusion. For adenovirus, it has been hypothesized that low pH activates one of the virion surface proteins to lyse the endosome membrane, releasing the virion into the cytoplasm (168). Thus, for enveloped viruses, this mechanism is similar to surface fusion, except that fusion of the viral envelope with the cell membrane occurs within the host cell. The main difference may be the pH, and thus the cellular site, at which fusion occurs.

Several experimental approaches may be used to distinguish these two pathways (49,137,239). First, electron-microscopic (EM) observation of newly infected cells may demonstrate a majority of virus particles being internalized in endosomes or undergoing fusion at the cell surface. The low specific infectivity (or high particle/plaque-forming unit ratio) for most animal viruses leaves open the possibility that virus particles may enter, or attempt to enter, the cell by nonproductive pathways. Thus, although this can be a very useful approach, EM studies can be misleading. Second, weak basic compounds, such as ammonium chloride, accumulate in acidic compartments of the cell, such as endosomes, and raise their internal pH. Because the pH drop is greatest within these organelles, the compounds inhibit the entry and infectivity of viruses through endocytosis more readily than that of viruses entering by surface fusion. Third, entry of viruses by endocytosis leads to internalization of viral surface proteins, whereas surface fusion leaves the virion envelope surface proteins on the cell surface as part of the plasma membrane. Thus, viral envelope proteins remain external and susceptible to protease digestion or reaction with antibodies after virus entry by surface fusion. Through experimental approaches such as these, these two pathways of virus entry can be distinguished.

Separate cellular functions may be needed for internalization versus release of viral nucleocapsids. As described above, HIV requires the CD4 molecule as a receptor. When the gene encoding CD4 was transferred into human and murine non-T cells, CD4 could bind to the cells and successfully infect some of the cells (133). However, some of the cell clones derived from murine cells could bind virus and internalize it, but none of the early events of infection ensued. Thus, there appear to be unique cellular products required after internalization of HIV for events such as release or uncoating of the virus.

Once within the host cell, the viral genome must be uncoated and transported to the correct intracellular site for transcription or replication. Many observations have led to a hypothesized role for the cellular cytoskeleton in transport of the genome to the nucleus or to the correct place in the cytoplasm (see below), but few precise mechanisms for transport are known.

VIRUS INTERACTIONS WITH THE CELLULAR TRANSCRIPTION APPARATUS

Viral messenger RNA (mRNA) is needed in the cellular replication of viruses in order to encode viral proteins needed for genomic replication and virion assembly. If the genomic RNA can be used as an mRNA directly (positive-strand virus), then synthesis of viral RNA need not precede initial rounds of translation. However, if the genomic nucleic acid is complementary to mRNA (negative strand) or in the form of DNA, *de novo* synthesis of viral mRNA must occur. If the virion contains an RNA polymerase, then synthesis of viral mRNA may depend on cellular factors, but only to a limited extent. However, if the virus uses cellular polymerases to synthesize mRNA, specific mechanisms may have evolved to promote transcription of viral DNA. In the following sections, I will examine ways in which viral infection alters host-cell transcription to facilitate the synthesis of viral RNA.

Inhibition of Cellular Transcription

Infection with many viruses leads to an inhibition of transcription of cellular protein-coding genes by host

RNA polymerase II. For RNA viruses, which do not use host-cell RNA polymerases for their replication, the presumed advantage conferred by this activity would be to provide higher pools of ribonucleoside triphosphate pools for viral RNA metabolism. For DNA viruses, inhibition of host transcription might allow the host-cell RNA polymerase II to transcribe the viral genome by decreasing competition for triphosphate precursors and transcription factors.

Little is known about how DNA viruses cause an inhibition of host-cell transcription, except for possible competition for RNA polymerase II and cell transcription factors. A possible mechanism for inhibition host transcription has been formulated for cells infected with the rhabdovirus, vesicular stomatitis virus (VSV). VSV infection causes rapid inhibition of host RNA synthesis, and this inhibition requires transcription of the viral genome (138,236). Ultraviolet (UV)-inactivation studies indicated that transcription of a small viral-encoded RNA called *positive-strand leader RNA* may be sufficient for inhibition of host transcription (71). The leader RNA accumulates in the cell nucleus early in infection (115) and associates with the cellular La protein (114) that binds transiently to nascent RNA transcripts (189). An oligodeoxynucleotide with a sequence identical to part of the leader RNA inhibited *in vitro* transcription of the adenovirus major late promoter and the VA RNA genes (72). A 65-kd HeLa cell protein binds to the oligonucleotide and can reverse the transcriptional block (73). Thus, this viral nucleic acid might bind a host-cell factor and prevent its binding to cellular promoters, thereby inhibiting host-cell transcription.

However, these experiments do not prove that the leader can inhibit transcription *in vivo*. Other workers have reported that viruses expressing very different amounts of identical leader RNAs can shut off host RNA synthesis equally well (44). Thus, there is no quantitative relationship between leader RNA and shut-off of host transcription. These studies do not prove or disprove a role for leader RNA in inhibition of host transcription, but they have raised the idea that viral gene products other than protein products can exert effects on host-cell metabolism.

Mechanisms of Stimulation of Cellular RNA Polymerase Activity

Packaging a Stimulatory Factor in the Virion

HSV encodes a protein that is assembled into the virion and, when introduced into the cell, becomes a part of a transcriptional activatory complex that specifically stimulates immediate early (α) gene expression (21,177). Studies examining the mobility of DNA–protein complexes during gel electrophoresis have shown that this protein can form complexes with host-cell proteins (140,178), notably the octamer transcription factor 1 (OTF-1); this factor binds to a sequence found in the α-gene promoters (62). It seems likely that one portion of the virion component binds to a DNA-binding protein (such as OTF-1) and that the other portion *trans*-activates gene expression (232). Thus, HSV provides for adequate transcription of its immediate early genes by bringing into the cell in its virion a protein that binds to a host cell, sequence-specific DNA-binding protein, causing increased transcription from immediate early viral gene promoters containing the specific sequence. In this way, a new complex (or a complex with increased activity) is formed by the addition of the virion protein.

Modifying Host-Cell Transcription Factors

Activity of host-cell RNA polymerases is increased after infection with several viruses such as adenovirus, herpes simplex virus, or pseudorabies virus. This is evident by induction of host-cell gene expression (153) or increased expression of other genes introduced by transfection (69,89). The mechanism of *trans*-activation has been most extensively studied with the adenovirus E1A gene product, the protein responsible for the increased polymerase activity after adenovirus infection (15,94). E1A increases the expression of polII-transcribed genes (as described above) and polIII-transcribed genes (12,61,80). The promoter requirements for E1A *trans*-activation coincide with basal-level promoters elements (14,154). Because E1A does not bind to DNA efficiently, it is believed that E1A increases transcription by affecting cellular transcription factors. In fact, there is evidence that E1A increases (a) the activity of the TFIIIC factor for polIII (80) and (b) the number or activity of specific polII factors (108,109,121,213,242). In the case of transcription factor TFIIIC, it has been hypothesized that activation by E1A involves phosphorylation of TFIIIC (81). Thus, E1A evidently increases the general activity of RNA polymerases by increasing the number or activity of probably several transcription factors, thereby increasing the activity of polII and polIII. In summary, the effect of E1A should be to increase the level of transcription of viral genes.

Induction or Expression of a New DNA-Binding Protein

In addition to expressing proteins that complex specifically with DNA-binding proteins, viruses may encode new DNA-binding proteins. For example, acute transforming viruses can encode nuclear oncogene products (see Chapter 13). As shown by predicted

amino acid sequence comparison and DNA-binding studies, one retrovirus encodes a homolog of the cellular transcription factor AP-1 (17). Thus, this retrovirus may directly affect transcription by encoding an altered form of a cellular transcription factor.

Thus, viruses may stimulate transcription in the infected cell by (a) encoding a transcription factor that directly binds to DNA, (b) encoding a protein that specifically interacts with a DNA-binding transcription factor, or (c) modifying the number or activity of cellular transcription factors.

VIRUS INTERACTIONS WITH RNA PROCESSING PATHWAYS

RNA splicing and transport to the cytoplasm are cellular pathways often utilized by viruses to mature their mRNA from nucleus to cytoplasm. In fact, the first evidence for RNA splicing came when the adenovirus late mRNAs were mapped on the viral DNA by R-loop hybridization (13,30). These studies showed that these mRNAs were encoded by noncontiguous regions of the genome. Splicing of the viral mRNA precursors is accomplished by cellular enzymes recognizing splice donor and acceptor sequences in the viral RNA. However, some viruses use the cellular splicing mechanisms but regulate the extent to which the full-length transcript is spliced. For example, influenza and retroviruses have transcripts that are infrequently spliced (see Chapters 21 and 27). In cells infected with influenza virus, the viral NS1 and M1 RNAs are spliced to yield the NS2 and M2 RNAs, respectively, at a frequency of 10% (119,120). Although splicing of the NS1 RNA is inefficient, formation of the spliceosome complex involving the snRNPs U1, U2, U4, U5, and U6 is efficient (2). Thus, the block seems to occur after formation of the spliceosome complex. The block may be mediated by the structure of the RNA itself or by a viral-encoded protein. This appears to be a situation in which virus infection regulates the extent of splicing of one of the viral RNAs, thereby regulating the levels of one of its own gene products.

Adenovirus inhibits maturation of cellular mRNA at a different stage. In adenovirus-infected cells, cell transcripts are synthesized and processed but do not accumulate in the cytoplasm (7). The adenoviral proteins E1B-55K and E4-34K are required for this effect on the host cell (4,74,172). Inhibition of host-cell mRNA transport would be likely to favor expression of viral proteins. However, it is uncertain how discrimination between host and viral mRNA occurs. This system may provide some important insights into the regulation and specificity of mRNA transport.

The regulation of RNA maturation may also provide a mechanism for temporal regulation of viral gene expression. HIV encodes several regulatory gene products from spliced mRNAs. One of these regulatory gene products, the *rev* gene product, stimulates the cytoplasmic accumulation of unspliced viral mRNAs that encode the viral structural proteins (50,103,134,199,217,227). Recent experiments have indicated that the *rev* gene product may promote the export of newly synthesized viral transcripts to the cytoplasm so that the splicing pathway is avoided (135). Thus, there may be cellular pathways that regulate (a) the assembly of splicing complexes or (b) the maturational pathway into which a newly made transcript enters.

Influenza virus intervenes in the host-cell mRNA maturation pathway in another novel way. Influenza mRNA transcription from the genomic RNA segments occurs in the host-cell nucleus (77). Host-cell nascent transcripts are cleaved by a virus-encoded endonuclease, and the 5′ end of the host transcript is used as a primer for synthesis of viral mRNA from the viral genome (19,174). Thus, influenza virus transcription complexes intervene in the host mRNA maturation pathway to obtain primer molecules for the viral transcription process.

VIRUS INTERACTIONS WITH THE TRANSLATIONAL APPARATUS

Once viral mRNA is available in the cytoplasm, it is translated by the host translational system to yield viral proteins. Many of the viral mRNAs are capped and contain a single major initiation site near the 5′ end. Thus, translation of these mRNAs is similar to that of host mRNA. In fact, much of the original evidence for the model for eukaryote ribosome scanning an mRNA for an initiation codon came from the identification and comparison of ribosome-binding sites on viral mRNAs (110). However, many other interactions of viruses with the host translational apparatus are possible, ranging from host shut-off to host defense against shut-off. The following sections provide a general description of the individual types of interaction between virus and the host translational apparatus. More details can be obtained in recent reviews (111,202).

Inhibition of Host Translation

After infection of the cells by many viruses, inhibition of host-cell mRNA translation occurs. Inhibition of translation of host-cell mRNA would provide the viral mRNA with increased availability of ribosomal subunits, translation factors, tRNAs, and amino acid precursors for protein synthesis. The extent to which shut-off of host translation is essential for efficient virus replication remains to be determined. There are

viable mutant viruses that are impaired in their ability to shut off host translation (see, e.g., refs. 16, 182, and 221). The *vhs*-1 mutant of HSV is somewhat impaired for growth as compared to wild-type virus (117,182), but the relationship between poor growth and limited host shut-off by this mutant virus remains to be demonstrated, due to the difficulty in performing genetics on this type of mutant.

Inhibition of translation of host-cell mRNA can occur by many mechanisms, and it is conceivable that individual viruses could utilize more than one of the following mechanisms.

Degradation of Host mRNA

After infection of cells by herpes simplex, poxvirus, or influenza virus, inhibition of host-cell translation occurs, and a decrease in the amount of intact host mRNA is observed (52,90,156,186). Thus, degradation of the host mRNA due to virus infection is one potential mechanism to provide free ribosomes and translational factors to preferentially translate the viral mRNAs. In some types of cells infected with herpes simplex virus, *de novo* viral protein synthesis is needed for degradation of host mRNA (156,157). In other cell types, a virion component can induce host polysome disaggregation and mRNA degradation (201). The virion host shut-off (*vhs*) mutants of HSV produce virions that are unable to inhibit host protein synthesis and degrade host mRNA (182,226). The *vhs*-1 mutation has been mapped within an open-reading frame of the HSV-1 genome (117), but the gene product has not been identified. The *vhs* gene product also destabilizes immediate early and early viral mRNA (116,162,163). Thus, there is no apparent discrimination between host and viral mRNA in this effect. In addition to providing a means for inhibiting host translation, this viral function could promote the shut-off of immediate early and early gene expression in the HSV lytic cycle (163).

Competition for the Host Translational Apparatus

Some viruses may not utilize specific effects on the host-cell translational apparatus to allow efficient synthesis of viral proteins. For example, it has been reported that VSV protein synthesis occurs preferentially in infected cells because (a) large amounts of viral mRNA compete for limiting ribosomes (130,205) or (b) the viral mRNA has higher affinity for ribosomes than cell mRNA (161). This is a controversial area because others have reported that (a) there is a specific viral gene function responsible for host translational inhibition (221), and (b) impairment of host translational factors eIF2 (26) or eIF3/4B (229) occurs in VSV-infected cells. There may be different mechanisms operating in different cells. Alternatively, more than one of the postulated mechanisms may be operating in one or more type of host cell. For example, a slight impairment of the host translational system would help switch translation from host mRNA to the more abundant or more efficiently initiating viral mRNA (129). Thus, more than one of the postulated mechanisms could be operating here.

Changing the Specificity of the Host Translational Apparatus

After poliovirus infection, translation of viral mRNA occurs and translation of host mRNA is inhibited. Extracts from virus-infected cells can translate poliovirus RNA but not host or VSV mRNA (192). Thus, the specificity of the translational apparatus appears to have been changed so that viral RNA is preferentially translated. Further analysis of the infected cell extracts has shown that the host translational component, cap-binding protein (CBP) complex, is inactivated in poliovirus-infected cells due to cleavage of one of the constituent proteins, the p220 protein (48). The CBP complex is needed for efficient initiation of translation of capped RNA (209). Poliovirus virion RNA is linked to a protein at its 5′ end (78,158), but the protein is removed in the cytoplasm so that polysomal RNA has pUp at its 5′ end (3). Thus, poliovirus infection inactivates the CBP complex so that translation of RNA not bearing capped 5′ ends can occur more efficiently. It has recently been shown that poliovirus 2A protease is required for cleavage of p220 (16), but it seems that this enzyme does not directly cleave p220 (128).

Initiation of translation can occur at an internal initiator codon on an mRNA molecule in poliovirus-infected cells, possibly by internal binding of the ribosomal subunits (169). This suggests that sequences within the untranslated 5′ region of poliovirus RNA can direct ribosome binding and initiation at internal sites within the RNA. Therefore, there are two mechanistic components involved in poliovirus conversion of the specificity of the translational machinery: (i) inactivation of the CBP complex so that initiation involving capped mRNA is decreased and (ii) a sequence within the poliovirus RNA that promotes use of internal initiation codons, possibly by ribosome binding internally within the RNA. This effectively results in the switch in translational specificity.

Host Response to Virus Infection

Among the host responses to viral infection is the synthesis of interferon (see Chapter 14). Interferon is secreted from the infected cell, binds to a second cell, and initiates a series of events, at least two of which

have an impact on the translation of viral mRNA. First, interferon activates an enzyme called the 2′,5′-oligosynthetase (100). The synthesis of 2′,5′-oligoadenylate by this enzyme activates a ribonuclease that degrades mRNA and rRNA (214). This effectively blocks viral mRNA translation. The second event is the induction of a kinase that phosphorylates the α subunit of the translation factor eIF-2 (146). This inactivates the translation factor and inhibits protein synthesis.

Viral Defense Against the Host Response

Some viruses, such as VSV and influenza, are very sensitive to interferon. Others, such as poxvirus and adenovirus, are relatively resistant to interferon. Vaccinia virus expresses a factor that inhibits the double-strand RNA-dependent eIF-2α kinase (164,187,238). Also, in vaccinia-infected cells, 2′,5′-oligoadenylate is produced, but the ribonuclease L is not activated (165,187). Thus, vaccinia virus seems to take active measures to prevent interferon-mediated inhibition of translation.

Adenovirus ensures efficient translation of late mRNA by encoding a small RNA known as VAI RNA (183). The VAI RNA is specifically required for late viral translation (203,228). The lack of VAI leads to an activation of the eIF-2α kinase and decreased eIF-2α activity (184,204,211). It is believed that the VAI RNA binds to the kinase and prevents dsRNA activation. Thus, VAI would block interferon effects on the host cell (101) or activation of the kinase by dsRNA molecules produced in infected cells, perhaps by symmetrical transcription.

Translational Frameshifting

In addition to the normal ribosomal protein synthesis mechanisms, some viruses exploit a potentially inherent ability of the host-cell ribosomes to shift from one reading frame to another during protein synthesis. During translation of the Rous sarcoma virus *gag* protein, approximately 5% of the ribosomes shift reading frames to synthesize a gag-pol fusion protein (91). Specific sequences in the RNA are required for the frameshifting (92). Therefore, specific viral RNA sequences cause ribosomal slippage so that small amounts of reverse transcriptase can be synthesized. This is one of the mechanisms used by viruses to express a limited amount of protein.

Suppression of Translational Termination

Expression of limited amounts of a viral protein can also be achieved by suppression of a nonsense codon and synthesis of a polyprotein. For certain retroviruses, this is the mechanism used to express the *pol* gene (107,171). Like frameshifting, this ribosomal effect requires *cis*-acting sequences on the viral mRNA (167). Again, viral nucleic acids direct cell proteins to perform certain activities that allow viral gene expression in regulated amounts.

VIRUS INTERACTIONS WITH THE CELL DNA REPLICATION APPARATUS

Inhibition of Host-Cell DNA Replication

Both RNA and DNA viruses cause the inhibition of host-cell DNA synthesis. The possible causes for this could be varied, as discussed below. The possible reasons for viral inhibition of cell DNA synthesis are (a) to provide precursors for viral DNA synthesis, (b) to provide host-cell structures and/or replication proteins for viral DNA synthesis, or (c) a secondary effect of inhibiting cellular protein synthesis. The possible mechanisms by which virus infection might inhibit cellular DNA synthesis are discussed individually.

A Secondary Effect of Inhibiting Cell Protein Synthesis

There appears to be a small pool of some essential cell DNA replication protein(s) because the rate of DNA chain growth decreases within minutes after inhibition of protein synthesis (173,225). It has been proposed that some viruses, such as the herpesviruses, inhibit cellular DNA synthesis as a consequence of inhibiting cellular protein synthesis (96). HSV and adenovirus DNA synthesis do not require the limiting cellular factor because their DNA synthesis continues independently of whether protein synthesis is ongoing or not (83,190).

Displacement of Cellular DNA from Its Normal Site of Replication

Herpesvirus infection has been variously reported to displace cellular DNA from the nuclear membrane (155) or to cause the displacement of cellular chromatin to the periphery of the nucleus (41,206). In either case, the cell DNA could be displaced from its normal location for replication because cell DNA synthesis has been reported to occur on the "nuclear cage" (139) or the nuclear matrix (11). The exposed sites on the nuclear matrix may provide a structural framework for viral DNA replication and late transcription (104).

Recruitment of Cell DNA Replication Proteins to Viral Structures

Recent studies have shown that herpes simplex virus infection leads to a redistribution of the host-cell DNA replication apparatus (42). This type of event could serve the dual function of providing cellular factors for viral DNA replication and also inhibiting cell DNA synthesis, thereby reserving deoxynucleotide triphosphates for viral DNA synthesis.

Degradation of Cellular DNA

Infection by vaccinia virus leads rapidly to a marked inhibition of cell DNA synthesis. A virion-associated DNase enters the host cell nucleus and acts on single-strand DNA (175,176). This inhibition is clearly mediated by the action of a virion component on the host cell.

Addition of One or a Few Viral Proteins to the Cellular Apparatus

Some viruses, such as the papovaviruses and parvoviruses, encode one or a few proteins which are inserted into the host-cell replicase complex and redirect the host-cell DNA polymerase to replicate viral DNA. For example, the SV40 large T antigen (a) binds the SV40 DNA sequences that serve as the origin of DNA replication (230), (b) forms a complex with the α-DNA polymerase (214), and (c) acts as a helicase (219). Through these and possibly other activities, T antigen promotes replication of the SV40 chromosome. T antigen could be viewed as an origin-binding protein that substitutes for a cellular protein. The ability of SV40 to replicate its DNA is evidently dependent on the interaction between T antigen and host-cell proteins, because SV40 DNA replication cannot occur in extracts prepared from certain types of cells (126). The interaction between T antigen and the polymerase α-primase complex seems to define the species-specificity (148). Therefore, the permissivity of a cell for viral growth can be defined by the ability of a viral DNA replication protein to interact with the cellular DNA replication apparatus.

Viruses Encoding a New Replicase Complex

Other viruses, such as adenovirus and herpesviruses, encode several proteins that form a major part of an infected cell-specific replicase complex. Adenovirus encodes a DNA polymerase, a terminal protein, and a DNA-binding protein (see Chapter 31). Cellular proteins also form a part of the replication complex. Herpes simplex virus encodes seven viral proteins required for viral DNA replication (28,241). Although the identity and role of specific cell proteins needed for HSV DNA synthesis have not been defined, the cellular DNA replication apparatus is redistributed after HSV infection, and at least part of it colocalizes with viral DNA replication proteins in the cell nucleus (42). HSV DNA replication proteins also anchor themselves onto the cell nuclear matrix (181), so several cellular proteins may be needed for optimal HSV DNA synthesis.

Viruses Encoding an Entire New Replication Apparatus

The poxviruses replicate entirely in the cytoplasm. Indeed, DNA synthesis can occur in enucleated cells. Therefore, they must encode all (or nearly all) of the proteins needed for replication of their DNA in the cytoplasm. Similarly, retrovirions contain an enzyme capable of copying the genomic RNA into DNA. This step is not possible in cells without the virion enzyme because a cellular reverse transcriptase is not available to copy the viral RNA. For these viruses, DNA synthesis is usually not restricted in different cells because the viral enzymes are viral-encoded.

Maintenance of Viral DNA Within the Host Cell

There are two types of mechanisms by which viral DNA is stably maintained within the host cell. First, retrovirus DNA is integrated into the cellular genome after its synthesis by the reverse transcriptase (see Chapter 27). Integration is promoted by the viral integrase function, but host functions can modulate the process. For example, the mouse *Fv*-1 gene mediates a postpenetration block to murine leukemia virus (MuLV) DNA integration (75,127). Thus, this host gene can define the host range of MuLV in mouse cells.

Second, viral DNA can be maintained as an extrachromosomal circular molecule in the infected cell. For example, Epstein–Barr virus DNA is maintained in latently infected lymphocytes as an episomal molecule (1) and requires a specific sequence, oriP, for replication and propagation of the genome in growing cells (245). Similarly, the bovine papillomavirus genome contains sequences (called *plasmid maintenance sequences*) needed to maintain and replicate the DNA as an extrachromosomal element (85,132). Replication of these viral DNA molecules requires *trans*-acting factors encoded by the viral genome and the host-cell DNA replication apparatus (see Chapters 30 and 35). In addition, these extrachromosomal elements appear to be subject to the normal copy number control existing in the normal cell because there is a constant

number of episomal copies of these viral DNA molecules per cell as the cells divide. Propagation and maintenance as an extrachromosomal element constitutes an additional way in which viral genomes can interact with the host cell.

VIRUS INTERACTIONS WITH CELL-PROTEIN MATURATION PATHWAYS

Maturation of viral proteins in infected cells involves mostly host-cell metabolic pathways including localization mechanisms, folding proteins, and enzymes that modify the primary translation product. Because viral proteins so often exploit the cell pathways, viral proteins have provided some very basic information about protein maturation pathways in the eukaryotic cell. However, exceptions exist where viral-encoded proteins themselves can affect protein maturation or modify the cellular maturation pathways. In the cases where cellular mechanisms are altered, this may be a source of cytopathology.

Utilization of Host-Cell Pathways

Protein Targeting Mechanisms

Eukaryotic cell proteins often contain specific signals that target the protein to a compartment or organelle within the cell. Individual viral proteins can contain all of the necessary signals for intracellular localization, because expression of the individual viral proteins in cells either by transfection or from a heterologous vector system leads to correct intracellular targeting of the viral protein (63,181,191,193,231). In other cases, more than one viral gene product is needed for efficient, correct localization (25,97,185,244).

Viral glycoproteins, especially the VSV glycoprotein (G) and influenza hemagglutinin (HA), have been used extensively as prototypes for the study of biogenesis of plasma membrane proteins. These proteins utilize the signal receptor particle, endoplasmic reticulum enzymes, Golgi apparatus enzymes, and transport mechanisms to realize their proper structure and cell-surface location. Part of the evidence for the notion that transmembrane proteins are synthesized on membrane-bound polyribosomes and translocated cotranslationally across the endoplasmic reticulum membrane while peripheral membrane proteins are synthesized on free polyribosomes came from the study of virus membrane proteins (105,147). Detailed studies of *in vitro* insertion of viral membrane proteins into the endoplasmic reticulum membrane came from studies of the VSV G protein (195). Detailed genetic study of the VSV G protein has provided evidence for the loop model for signal sequence insertion into the endoplasmic reticulum (210).

Similarly, viral nuclear proteins utilize cellular pathways to enter the nucleus. In fact, a nuclear localization signal was first identified in a viral protein (95).

The need for proper protein folding and assembly for correct intracellular localization has become apparent from studies of viral proteins. For example, correct folding and trimerization are needed for the influenza hemagglutinin (34,35,43,64,113) to localize from the endoplasmic reticulum to the Golgi apparatus. Folding and assembly of the proteins were monitored by (a) reactivity of the proteins with conformation-specific monoclonal antibodies, (b) protease sensitivity, and (c) sedimentation of proteins and complexes on velocity sedimentation gradients. Localization was monitored by cell fractionation and immunocytochemistry. These studies also showed that mutant viral proteins or improperly folded wild-type proteins coprecipitate in immunoprecipitates with a cellular protein induced under stress conditions (64,207). This cellular protein, called BiP or grp78, is a member of the hsp70 family of proteins. It has been proposed that members of this family of proteins recognize incompletely or improperly folded proteins, catalyze their unfolding, and help them to attempt to fold into the correct conformation (170). It has also been proposed that BiP might prevent abnormally folded proteins from being secreted and exposed to the immune system, thereby increasing the repertoire of epitopes recognized as "self" (64). The viral glycoproteins provide systems to study further the role of these cellular "chaperone" proteins in folding of normal proteins and in the metabolism of abnormal proteins.

Protein Modification

Many of the posttranslational modifications of proteins that occur in cells (i.e., cleavage, glycosylation, phosphorylation, acylation, or sulfation) are performed by cellular enzymes. Most of these enzymes are ubiquitous, and thus their presence is not usually limiting for viral replication in different cell types. One example of an exception to this is the presence of tissue-specific proteases that cleave specific virion surface glycoproteins, allowing the viral particles to become infectious (200).

Some protein modifications in infected cells are the result of the direct action of viral-encoded gene products. Some viral proteins are protein kinases, such as the v-src protein and related oncogene proteins (32,125). For the oncogene proteins, the major target proteins of the kinase activity are cellular proteins (87). Also, some viral proteins can act as proteases to make specific protein cleavages not readily made by cellular

enzymes (112), such as the picornaviral and retrovirus proteases.

Cellular protein modification activities can also be modified by viral infection. The polyoma virus *hr-t* gene function is required for transformation of nonpermissive cells (8) and for proper assembly of polyoma virions (58). In cells infected with *hr-t* mutants altered in the middle T-antigen gene, phosphorylation of the polyoma major capsid protein is reduced. The polyoma middle-T antigen is known to bind to the cellular c-src protein and stimulate its tyrosine kinase activity (36). This kinase may phosphorylate another protein kinase, with the ultimate effect being an increased phosphorylation of VP-1. Thus, this alteration in the cellular enzyme activity may be necessary for efficient assembly of infectious virus particles.

EFFECTS OF VIRUSES ON CELL STRUCTURE

Effects of Viruses on the Cell Membrane

Viruses can alter the membranes of their host cell in at least two ways: (i) by promoting membrane fusion with neighboring cells and (ii) by altering the permeability of the cell plasma membrane. Both of these effects may be exerted through the insertion of viral encoded proteins within the membrane.

Promotion of Cell Fusion

Some enveloped virions have cell-surface proteins that facilitate fusion of the virion envelope with the cell-surface membrane. This property can confer on the virion the ability to promote fusion between adjacent cells. For example, in sufficient amounts, Sendai virus can bind to, and cause fusion between, two neighboring cells, leading to a polykaryon. In addition, viral glycoproteins expressed within the infected cell can migrate to the cell surface and promote fusion with neighboring cells. This latter phenomenon has been referred to as *fusion from within* to distinguish it from fusion of adjacent cells caused by input virions, *fusion from without* (20).

The induction of cell fusion may be a form of cytopathology that is a side product of the membrane fusion activity that allows entry of the virus at the cell surface. It is not clear whether the fusion of the neighboring cells is necessary for cell-to-cell spread of virus.

Altering Plasma Membrane Permeability

Infection by viruses may cause an increase in permeability of the host-cell plasma membrane to ions, allowing, for example, an influx and increase of intracellular sodium ions. Because the translation of some viral mRNAs is more resistant to high sodium ion concentration than translation of cell mRNA (24), it has been hypothesized that this may favor translation of viral mRNAs. Indeed, increased osmolarity of the culture medium inhibits host-cell translation (198,237) and allows preferential translation of viral RNAs (32,160). For Sindbis virus, the increase in permeability seems to correlate temporally with shut-off of host protein synthesis (59), but membrane permeability changes occur later than host shut-off in cells infected with picornaviruses (45,118,151). Furthermore, changes in ion flux do not account for host shut-off by certain other viruses (51,68).

Increased membrane permeability to antibiotics and toxins in infected cells has also been reported (33,53). This was suggested as a possible antiviral strategy to kill infected cells (23).

The cause(s) of the permeability changes in infected cells has not been defined. Insertion of viral proteins into the membrane was hypothesized to alter the permeability of the plasma membrane (23). RNA virus mutants unable to transcribe viral mRNA failed to alter the membrane permeability (99). Therefore, viral gene products expressed in the infected cell are necessary for the membrane changes, but no specific genetic defects have defined individual gene products required for altering the cell membrane permeability.

One aspect of the mechanism of the change in permeability was studied by Garry et al. (60). They hypothesized that Sindbis virus might inhibit the sodium pump in the plasma membrane which maintains the ionic balance within the cell. When they added ouabain (an inhibitor of the sodium pump) to uninfected cells, protein synthesis was inhibited. However, when ouabain was added to Sindbis virus-infected cells, viral protein synthesis was not affected. In addition, the Na^+ and K^+ concentrations did not change in infected cells upon ouabain treatment. These results indicate that Sindbis virus infection has an effect on cells similar to ouabain treatment. Therefore, for some viruses, increased membrane permeability may be a form of CPE that allows preferential biosynthesis of viral gene products.

Interactions Between Viruses and the Cytoskeleton

The cell cytoskeleton plays several roles in (a) the structure of the cell and (b) the transport and movement of organelles. Therefore, it should not be surprising that there are various associations between

viral macromolecules and the cellular cytoskeleton in the infected cell.

Depolymerization of Cytoskeleton Filaments

Infection by many viruses leads to a disruption of one or more cytoskeletal fiber systems. For example, infection of cells by several viruses, including VSV (79,145,197), vaccinia virus (79,145), simian virus 40 (67,196), canine distemper virus (84), frog virus 3 (149), and HSV (6,76,240) cause a decrease in actin-containing microfilaments. Many of these studies have used immunofluorescence as an assay for microfilaments (see Fig. 4). Some studies have used DNase I inhibition as a quantitative assay for globular actin as a measure of actin filament depolymerization (see, e.g., ref. 6). The mechanism by which this disassembly occurs has not been defined, but expression of HSV immediate-early and early proteins seems to be necessary and sufficient for microfilament depolymerization (76). Many of these viruses, including HSV, canine distemper virus, and frog virus 3, also cause a depolymerization of microtubules in infected cells. In contrast, infection of cells by reovirus causes disruption of vimentin-containing intermediate filaments but spares the microfilaments and microtubules (208). Although the cause of the cytoskeleton changes is not clear, it is clearly a potential cause of the structural changes that occur in infected cells, such as cell rounding, because the major cytoskeletal fibers play a role in maintaining cell morphology. The cytoskeletal changes may not be primary effects of viral replication. Instead, other effects of virus infection, such as inhibition of

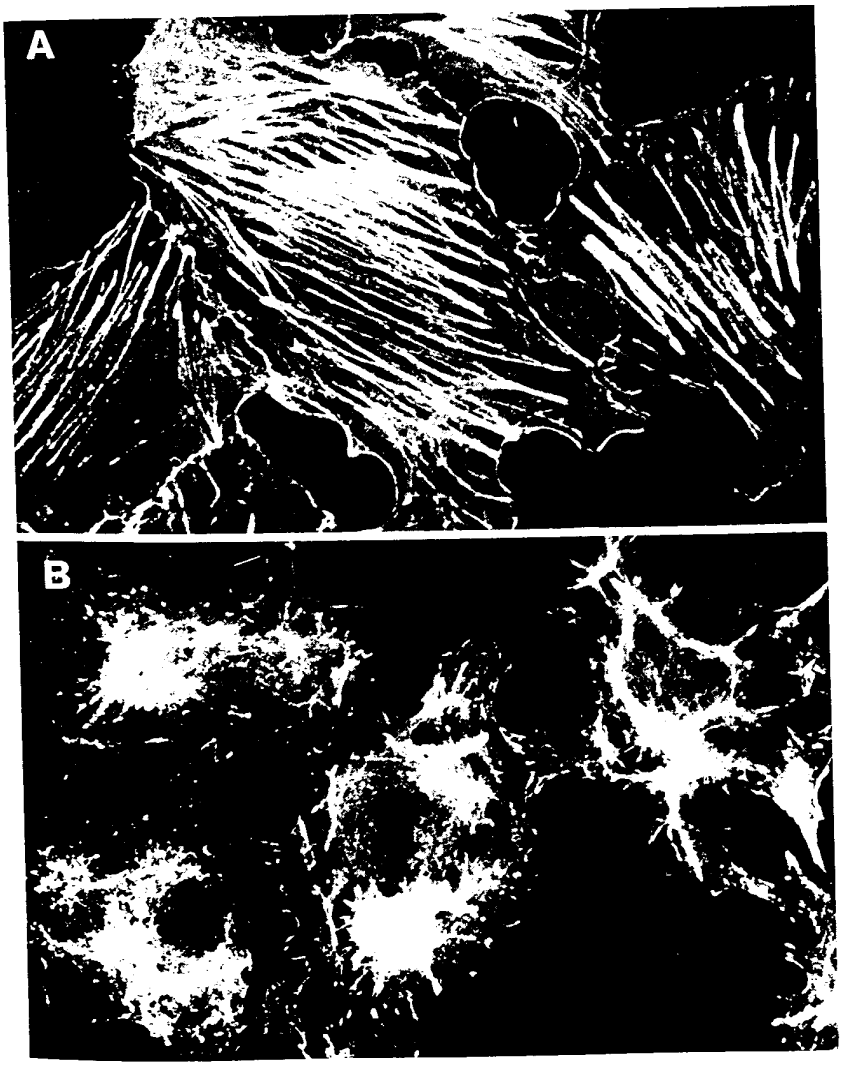

FIG. 4. Disruption of the cytoskeleton by virus infection. Uninfected (**A**) and HSV-infected (**B**) monkey cells were fixed, permeabilized, and stained with fluorescein-conjugated phalloidin. Phalloidin reacts with filamentous actin (f-actin) and thus reveals the distribution of microfilament bundles. (Micrographs courtesy of S. Rice.)

macromolecular synthesis, might lead to cytoskeletal changes.

Incorporation of Cytoskeletal Components into Infected Cell Structures

As described below, new structures called *factories* or *inclusions* are assembled in the nucleus or cytoplasm for synthesis of viral nucleic acids and assembly of virions. In reovirus-infected cells, there is evidence that a specific cytoskeleton component is incorporated into the cytoplasmic inclusion bodies. EM studies showed that the inclusions contain several types of filament (37,39). These include microtubules and 50- to 80-Å "kinky" filaments. Dales et al. (39) postulated that the 50- to 80-Å filaments represented cellular filaments reorganized into the cytoplasmic factory. Sharpe et al. (208) showed that anti-vimentin antibody stained filaments in inclusions and that these may therefore be the cellular filaments reorganized within the inclusions. Thus, viral infection may exploit cellular structural elements to build replication factories.

Interpretation of some immunofluorescence data has been complicated, however. For example, anti-actin monoclonal antibodies can stain intranuclear replication compartments in cells infected with HSV (S. A. Rice and D. M. Knipe, *unpublished results*). However, the anti-actin antibodies cross-react with the HSV immediate early ICP4 protein molecule on Western blots. Although this could be an example of molecular mimicry (57) and could have some functional significance, the cross-reaction could be fortuitous. Most importantly for this discussion, the immunological cross-reaction of anti-actin monoclonal antibodies with ICP4 makes it difficult to localize actin by immunofluorescence after HSV infection.

Interactions of Viral Molecules and the Cytoskeleton

The cellular substructure—the cytoplasmic cytoskeleton, nuclear matrix, and membranes—provides a physical site and possibly some functional elements for many metabolic activities of the cell. The cytoskeleton provides (a) a substrate for polyribosomes, (b) structural integrity for the cell, (c) a structural framework for organelle movement, and (d) part of a system for cellular movement. The nuclear matrix provides a substrate for transcription and DNA replication complexes. Therefore, it is not surprising that many viral macromolecules are associated with the cytoskeleton. Using gentle detergent extraction procedures, the following have been shown to fractionate preferentially with the cytoskeleton and nuclear matrix: SV40 newly uncoated genomes, nascent RNA molecules, and RNA in transit from cytoplasm to nucleus (9,235); adenovirus DNA (246) and HSV proteins in transit from cytoplasm to nucleus (10,180); and VSV proteins during nucleocapsid assembly (29). Thus, it is likely that part of the subcellular compartmentalization of viral processes relies on the structural organization of the host cell. In addition, the virus provides for part of the compartmentalization by specifying the assembly of infected cell-specific structures, such as the replication factories described below.

Certain viral components have been shown to undergo specific associations with the cytoskeleton. For example, electron microscopy has shown adenovirus to be associated with microtubules in infected cells, an association believed to reflect nuclear transport of the parental virus (38). Adenovirus virions also can bind to microtubules *in vitro* (131). Similarly, reovirus particles are associated with microtubules in infected cells (37), and reovirus particles bind to microtubules *in vitro* (5). Also, viral proteins and viral factories codistribute with microtubules (5). Interestingly, colchicine does not lower virus yields but inhibits formation of large cytoplasmic inclusions (218). The cytoskeleton may also play a role in virus assembly, because disruption of portions of the cytoskeleton can block budding of some enveloped viruses (166,220). In general, the cytoskeleton is thought to play a role in providing a structure for viral replication or movement of macromolecules, but the precise molecular mechanisms remain to be defined.

Effects of Viruses on mRNA Association with the Cytoskeletal Framework

Using a gentle detergent extraction procedure to isolate the cytosketal elements, Lenk et al. (123) showed that polyribosomes were preferentially associated with the cytoskeleton fraction of the cell. Cervera et al. (27) showed that VSV mRNAs were also associated with the cytoskeleton fraction while being translated. These observations led to the hypothesis that cytoskeleton association was necessary for translation of mRNA. As discussed above, viruses cause a disruption of portions of the cytoskeleton. Lenk and Penman (122) showed that poliovirus disrupted the cytoskeleton of HeLa cells and caused a release of host mRNAs from the cytoskeleton.

It was also reported that adenovirus infection of human KB cells caused a dissociation of host mRNA from the cytoskeleton (234). The correlation between shut-off of host translation and dissociation of mRNA suggested that dissociation of host mRNA might be a general mechanism for virus inhibition of host translation. However, other reports have indicated that VSV (18) and adenovirus and influenza virus (98) inhibit host translation but do not cause a dissociation

of host mRNA from the cytoskeleton. Poliovirus dissociation of host mRNA from the cytoskeleton has been confirmed (98). Thus, dissociation of the host mRNA from the cytoskeleton is not required for viral inhibition of host translation. Poliovirus may cause a more drastic rearrangement of the cytoskeleton (98,122) than the other viruses; or by some other effect, such as cleavage of the P200 protein of the CBP complex (see above), poliovirus may destabilize the normal association between the CBP components and the cytoskeleton (247). Therefore, the dissociation of host mRNA from the cytoskeleton in poliovirus-infected cells may be related to either (a) host shut-off or (b) a secondary effect of another aspect of poliovirus infection. If the two events are related, the study of poliovirus-infected cells could give further insight into the role of cellular factors and the cytoskeleton in protein synthesis.

Assembly of Factories for Nucleic Acid Replication and Virion Assembly

Viral replication proteins and assembled virions often accumulate in specific regions of the nucleus (e.g., inclusion bodies or replication compartments in cells infected with herpesviruses or adenovirus) or cytoplasm (e.g., the Negri body in rabies-infected cells). The assembly of these new structures in the infected cell often displaces host-cell components from specific regions of the cell and leads to one form of CPE.

The inclusion bodies, or areas of altered staining in infected cells, are useful in diagnostic virology because they are found at locations in the cytoplasm or nucleus (or both) which are characteristic of specific groups of viruses. Also, they show characteristic staining properties in that they are basophilic or acidophilic. A more detailed consideration of the inclusion bodies formed by one virus, HSV, illuminates several important aspects of this form of cytopathology. First, the development of nuclear inclusions in HSV-infected cells goes through several stages. Second, the analysis of these inclusion exemplifies several approaches to the study of virus interactions with the host cell.

Electron microscopy of herpesvirus-infected cells has revealed electron-translucent intranuclear inclusions surrounded by marginated and compacted cell chromatin (41,206) (Fig. 5). Light-microscopic observation of nuclear inclusions reveals an hourglass ap-

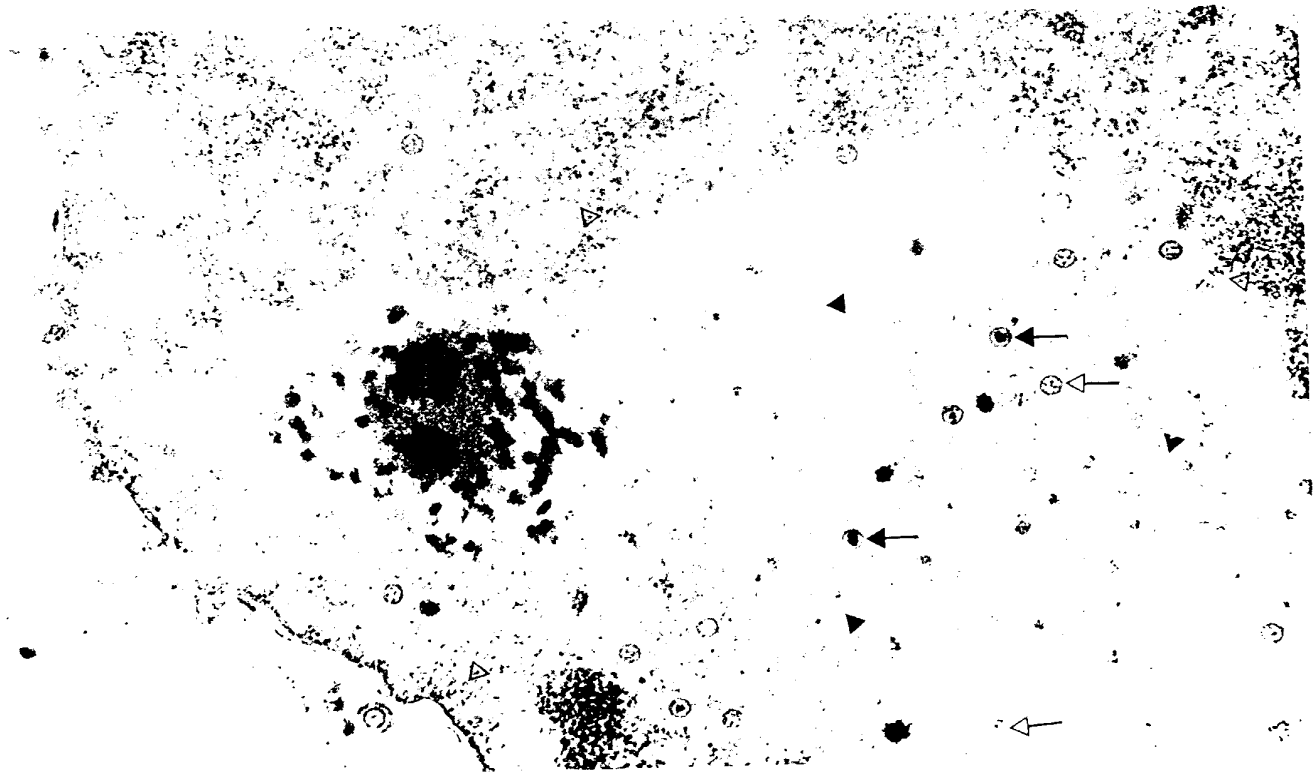

FIG. 5. Electron-microscopic visualization of nuclear inclusion areas. The *filled triangles* denote the electron-translucent nuclear inclusion area in a human HEp-2 cell infected with HSV-1. The *filled arrows* indicate full capsids, and the *unfilled arrows* indicate empty capsids. The *unfilled triangles* indicate host chromatin compressed to the periphery of the nucleus, apparently by the nuclear inclusion. (Micrograph courtesy of D. Furlong and B. Roizman.)

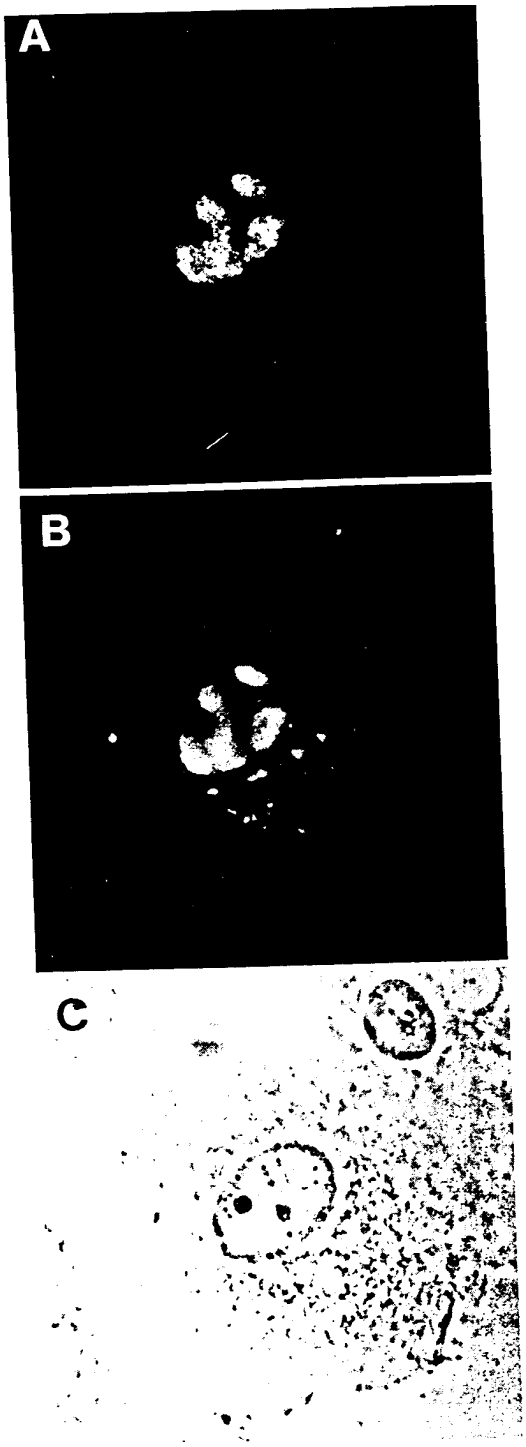

FIG. 6. Codistribution of a DNA replication protein and a transcriptional activator protein by double-label immunofluorescence. Monkey (Vero) cells infected with HSV-1 were fixed with formaldehyde, permeabilized with acetone, and reacted with the anti-ICP8 39S mouse monoclonal antibody and anti-ICP4 rabbit serum followed by rhodamine-conjugated goat anti-mouse immunoglobulin antibody and fluorescein-conjugated goat anti-rabbit immunoglobulin antibody (as in ref. 106). **A**: Rhodamine fluorescence, showing the distribution of the HSV DNA replication protein ICP8. **B**: Fluorescein fluorescence, showing the distribution of the transcriptional activator protein ICP4. **C**: Phase-contrast micrograph of the same field.

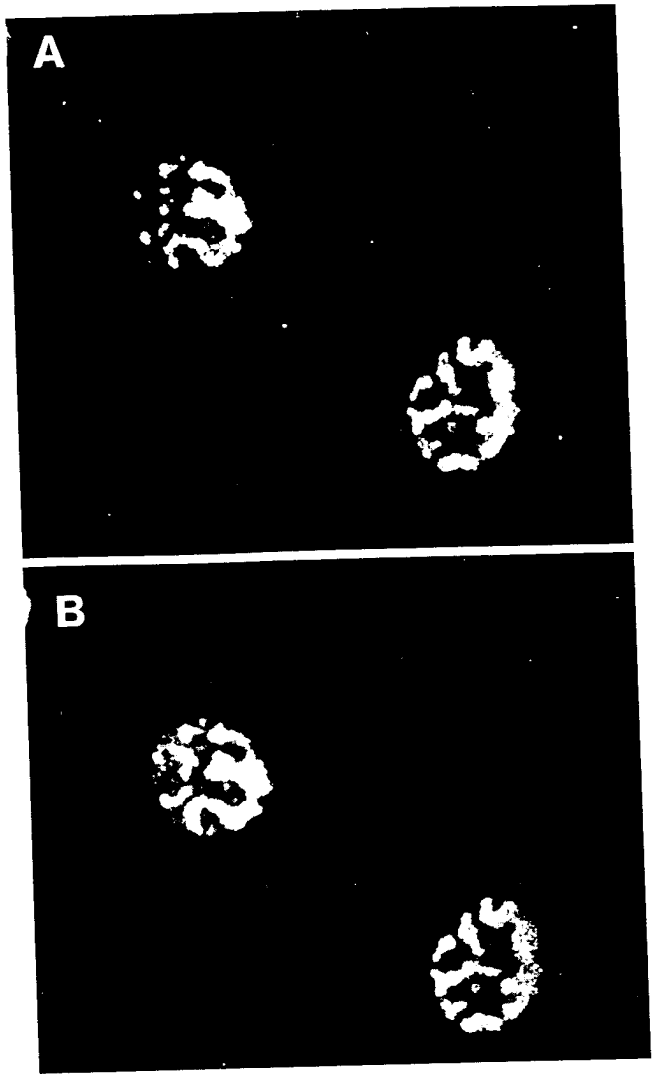

FIG. 7. Immunofluorescence detection of sites of nucleic acid synthesis in virus-infected cells. Monkey (CV-1) cells infected with HSV were pulse-labeled for 15 min with bromodeoxyuridine (BrdU), an analogue of thymidine. The cells were fixed, permeabilized, and reacted with mouse anti-BrdU monoclonal antibody and rabbit anti-ICP8 serum followed by rhodamine-conjugated goat anti-mouse immunoglobulin antibody and fluorescein-conjugated goat anti-rabbit immunoglobulin antibody (as in ref. 42). **A**: Fluorescein fluorescence showing the distribution of the HSV DNA replication protein ICP8. **B**: Rhodamine fluorescence showing the distribution of DNA synthesis sites. (Micrographs courtesy of A. de Bruyn Kops.)

pearance of the inclusions at early times and an eosinophilic staining at later times (150,216) (Fig. 3). Immunofluorescence experiments using antibodies specific for HSV DNA replication proteins have shown that viral DNA replication proteins accumulate in intranuclear foci by 3 hr post-infection and that these foci enlarge into globular nuclear structures called *replication compartments* (42,181) (Fig. 6). The replication compartments are likely to be equivalent to (a) the translucent nuclear inclusions seen by electron microscopy (Fig. 5) and (b) the early nuclear inclusions seen by light microscopy. Bromodeoxyuridine pulse labeling followed by immunofluorescence detection of BrdU-substituted DNA has shown that viral DNA synthesis occurs in the replication compartments (42) (Fig. 7). This technique allows the determination of the cellular location of DNA synthesis. Similarly, *in situ* hybridization with a viral DNA probe has shown that progeny viral DNA accumulates in replication compartments (Fig. 8).

The accumulation of progeny DNA [the probable template for late gene transcription; see Fig. 8 and the viral transcriptional *trans*-activator protein ICP4 (106) (Fig. 5)] in replication compartments suggests that late gene transcription occurs in the replication compartments. Thus, late gene transcription may be compartmentalized in infected cell-specific nuclear structures. This may provide a system to study the mechanisms involved in compartmentalization of transcription within the cell nucleus.

Empty capsids may be assembled around dense bodies or in the inclusions within the infected cell nucleus (Fig. 5). Encapsidation of viral DNA appears to occur within the inclusion body itself (Fig. 5). Crystalline arrays of capsids and nucleocapsids may accumulate in inclusions in HSV-infected cells or in cells infected with other viruses. Thus, these intranuclear structures in herpesvirus-infected cells may be involved in the processes of DNA replication, late gene transcription, and nucleocapsid assembly.

RELEASE OF PROGENY VIRUS

The mechanism of release of progeny virus from the infected cell depends on the structure of the virus. Enveloped viruses exit from the infected cell either by budding through the plasma membrane (Fig. 9) or by fusion of secretion vesicles containing virus particles with the plasma membrane (223). Thus, nucleocapsids can bud through the plasma membrane (orthomyxoviruses, paramyxoviruses, rhabdoviruses, and retroviruses), directly producing extracellular virions, or through internal membranes such as the endoplasmic reticulum (ER) (rotaviruses), ER and/or Golgi apparatus (coronaviruses and bunyaviruses), or inner nuclear membrane (herpesviruses). The factors that determine the site of budding of a virus are not well understood, but the site of localization of the surface glycoproteins must be one important factor.

Polarized epithelial cells have differentiated apical and basal surface plasma membranes. Thus, viruses budding through the plasma membrane or vesicles containing virus particles can traffic specifically through either membrane. For example, orthomyxoviruses and paramyxoviruses bud at the apical surface, whereas VSV and retroviruses bud at the basal surface. Viral glycoproteins have an intrinsic ability to localize to specific surfaces of polarized cells (see, e.g., refs. 93, 179, 193 and 224), and this may be an important factor in deciding the site of virus budding in polarized epithelial cells. Sorting of proteins destined for the dif-

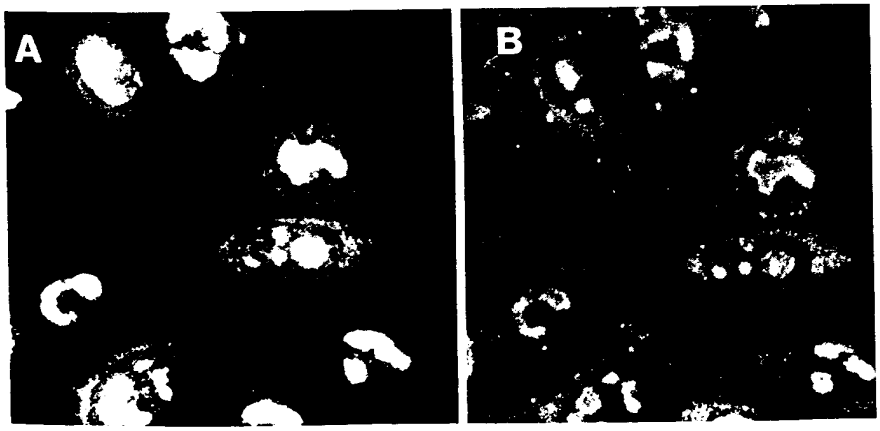

FIG. 8. *In situ* hybridization detection of the location of viral nucleic acids in infected cells. Monkey cells infected with HSV-1 were fixed in formaldehyde, permeabilized in acetone, and incubated with biotin-labeled HSV DNA. The cultures were heated to denature the cellular DNA and the probe, and the culture and solution were allowed to cool. The cells were then reacted with mouse anti-ICP4 monoclonal antibody followed by rhodamine-conjugated goat anti-mouse immunoglobulin antibody and fluorescein-conjugated avidin. **A**: Rhodamine fluorescence showing the location of the HSV *trans*-activator protein ICP4. **B**: Fluorescein fluorescence showing the location of HSV DNA in the infected cells. (Micrographs courtesy of S. Rice.)

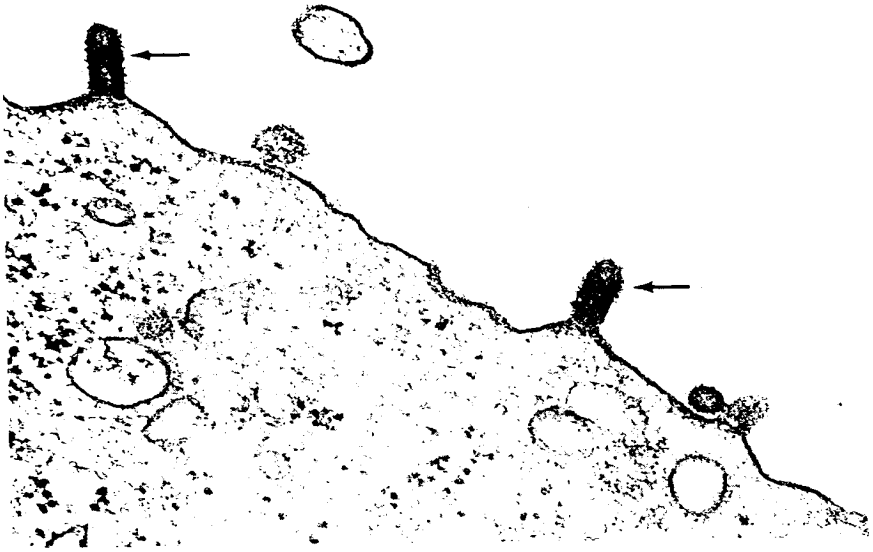

FIG. 9. Enveloped virus particles budding from the infected cell surface. The *arrows* indicate VSV particles budding from the surface plasma membrane of infected Chinese hamster ovary cells, as visualized by electron microscopy.

ferent surfaces appears to occur within the Golgi apparatus (188). Studies of the targeting of chimeric glycoproteins expressed from recombinant clones have yielded conflicting results. Some studies have indicated the ectodomain of the glycoprotein as containing the targeting signal (142,194), whereas other studies have not found the ectodomain sufficient to determine the site of localization (179,223).

It has been generally assumed that unenveloped viruses are released by lysis of the cells but that some unenveloped viruses may exit from the cell without cell lysis (159), and one report indicates polarized release of SV40 from epithelial cells (31). Thus, there may be cellular mechanisms utilized by viruses for the active release of unenveloped viruses which are not the result of lysis of the host cell.

Once the progeny viruses have been released, they can initiate infection in new cells, and a whole new round of virus replication and interaction with a host cell can begin.

SUMMARY

The major points of this chapter can be summarized as follows:

1. The ability of a virus to replicate in a host cell can be determined by the availability of specific host macromolecules in the host cell. These molecules may be external, such as a receptor, or internal, such as a transcription or replication factor.
2. Some of the cytopathic effects of virus infection on a host cell are due to specific alterations in host-cell metabolism or structure that allow viral replication events. These cytopathic effects are not simply toxic side effects of virus infection.
3. Interactions of viruses with host cells may involve subtle changes in the host cell, and understanding of the nature of the interaction between viral gene products and the host-cell molecules provides insight into the workings of the host cell.

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